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Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses?

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1. INTRODUCTION

Harris[108] first postulated the existence of hypothalamic hormones that could be released from the median eminence of the hypothalamus to trigger the secretion of hormones from the adenohypophysis. These 'hypophysiotropic hormones' are more commonly known as releasing factors because of their critical role in releasing adenohypophyseal hormones. In 1955, Saffran and Schally[201] and Guillemin and Rosenberg[102], independently provided the first convincing demonstrations of the existence of a factor derived from the hypothalamus that could elicit adrenocorticotropin (ACTH) secretion from the pituitaries of intact rats (for reviews, see Refs. 202 and 267). The factor was named corticotropin-releasing factor (CRF) because of its ability to stimulate secretion of ACTH. Although CRF was the first of the hypothalamic-releasing factors to be named, the elucidation of its structure was fraught with exceptional technical difficulties[202,267] and the structures of several other hypothalamic releasing factors were determined earlier. Finally in 1981, Vale and his colleagues succeeded in determining the structure of ovine CRF (oCRF)[246]. Jean Rivier synthesized the compound according to the postulated structure and Vale's group showed that it had biological activity identical to that of the purified natural hormone[246]. The sequence of the hormone has now been determined in sheep, man, rats, pigs, goats and cows, and in all cases contains 41 amino acids and a similar primary structure (for a review, see Ref. 187). There is now little, if any, dispute that the 41-amino acid polypeptide synthesized by Vale et al. is the major endogenous corticotropin-releasing factor, although a number of other factors (e.g. vasopressin (VP), catecholamines) are known to participate in the regulation of ACTH release[8,80,187].

The availability of purified synthetic CRF and antibodies to it has enabled study of the biological activities of CRF. Histological studies with CRF antisera have indicated that CRF-like immunoreactivity (irCRF) exists in neurons outside the hypothalamus and that hypothalamic neurons send axons to regions of the brain other than the median eminence region[208,209]. In addition, binding sites for CRF have been found in a distribution similar to irCRF. Neurochemical studies have indicated a Ca^{2+}-dependent release of CRF stimulated by K^+(Ref. 224). Moreover, CRF has been shown to stimulate adenylate cyclase activity in slices of brain tissue, just as it does in the pituitary[2,48,244,262]. Electrophysiological studies have shown regionally specific responses to iontophoretically applied CRF[76]. Together, these results provide a strong basis for postulating a neurotransmitter role for brain CRF.

Administration of CRF to animals has indicated that the molecule has a variety of endocrine, physiological, neurochemical and behavioral activities that are not shared with ACTH or corticosterone. These observations have suggested a role for CRF beyond that of the regulation of ACTH release. Investigators in La Jolla (including Vale, Rivier, Brown, Fisher, Swanson and Koob) recognized that many of the effects of CRF resembled those observed in stress, suggesting the possibility that CRF may be an endogenous mediator of such responses[133]. The purpose of this review is to survey the

reports of administered CRF and to assess what role(s) cerebral CRF may play beyond that of an initiator of the hypothalamic-pituitary-adrenal (HPA) axis. Specifically, we shall examine the evidence for a role of cerebral CRF in stress.

2. THE CEREBRAL DISTRIBUTION OF CRF AND BINDING SITES FOR CRF

The distribution of CRF and CRF-binding sites in the brain has been described and reviewed by others[158,204,231]. Although it is beyond the scope of this review to discuss this distribution in detail, a brief summary of the major findings will be presented.

2.1. The cerebral distribution of CRF

The paraventricular nucleus of the hypothalamus (PVN) is the primary source of CRF released from the median eminence into the portal blood supply, although most hypothalamic nuclei contain some irCRF and send minor projections to the median eminence[158,209,231]. However, CRF-like material and specific, high-affinity binding sites have been identified in many extrahypothalamic regions of the CNS. A few CRF-positive fibers from the PVN project to brain stem autonomic nuclei[208]. Identification of CRF-like material within the CNS has involved both immunohistochemical methods[158,161,231] and the combined use of HPLC purification and bioassay measuring the secretion of ACTH from anterior pituitary cells in vitro[166]. Although both techniques have indicated a similar pattern of localization of CRF within the brain, the bioassay has not been used to study localization in as fine detail as that provided by immunostaining techniques. The importance of verifying CRF localization using techniques other than those based on recognition by antisera or at least using antisera with different specificities, is emphasized by a report that an antiserum to CRF also recognized substance P (ref. 15).

In general, irCRF is found in areas of the limbic system, structures involved in regulating the autonomic nervous system and regions associated with the processing of sensory information in both rats and primates. Within the telencephalon, the greatest number of irCRF-containing cell bodies was found in the prefrontal, insular and cingulate cortices[158,231]. A third study observed a more uniform distribution within the cortex[204]. IrCRF-containing perikarya were localized primarily to layers II–III with processes extending through layers I–IV. Other regions that contain relatively high concentrations of irCRF include the central nucleus of the amygdala, the olfactory bulb, certain thalamic nuclei, the substantia nigra pars compacta, the periaqueductal grey, the locus coeruleus (LC), the nucleus of the solitary tract, the dorsal and ventral parabrachial nuclei and the cortex and deep cerebellar nuclei of the cerebellum[86,158,204,208,231]. The relative concentration of irCRF detected in different brain structures varies in the different studies. This is probably accounted for by differences in the species studied, the antisera used and whether or not the animals were pretreated with colchicine.

Although the projection patterns of irCRF-containing neurons have not been described in detail, projections from the amygdala to the parabrachial nucleus[161], from the inferior olivary nucleus to the cerebellum[46,56,186] and from the perifornical and anterior hypothalamic areas to the lateral septum[205] have been observed. The localization of CRF within limbic and autonomic structures provides an anatomical basis for the participation of CRF in coordinating visceral and behavioral responding.

2.2. The cerebral distribution of binding sites for CRF

Using both quantitative autoradiography and cell-membrane binding techniques, high-affinity binding sites for CRF have been observed in a pattern similar to that observed for irCRF[63,262]. A high density of CRF-binding sites was observed throughout neocortex with layers I and IV containing the greatest density of sites. Outside the CNS, specific CRF-binding sites have been located in all sympathetic ganglia, chromaffin tissue of the adrenal medulla and in other organs of the body, including the gut, pancreas and spleen[244].

3. NEUROCHEMISTRY OF ENDOGENOUS AND ADMINISTERED CRF

3.1. Release of cerebral CRF

Evidence for a neurotransmitter role for CRF in the brain is provided in part by the anatomical evidence discussed above for parallel distributions of immunoreactive and bioactive CRF and of CRF-binding sites. In addition, there is evidence for a neurotransmitter-like release of CRF from brain tissue. Suda et al. observed release of irCRF from perifused rat hypothalami in vitro[229]. The release induced by K^+ was completely dependent on the presence of Ca^{2+} in the incubation medium. Subsequently, Smith et al. demonstrated a similar phenomenon for extrahypothalamic regions of the brain[224]. Minced brain tissue from the amygdala, midbrain, and striatum, like that from hypothalamus, was shown to release irCRF following stimulation with 56 mM K^+ or scorpion venom, in a manner requiring Ca^{2+}. No such effect was observed with cerebellar tissue. These results thus provide some basis for postulating a neurotransmitter function for brain CRF.

Evidence for a functional role of cerebral CRF is provided by reports of changes in the cerebral concen-

trations of CRF in various brain regions following stressful treatments. A preliminary report indicated a doubling of CSF concentrations of irCRF following 15 min footshock in rats[29]. Chappell et al. studied irCRF in 32 brain regions of the rat following 3 h of cold restraint (acute stress) or a 13-day series of different 'unpredictable' stressors (chronic stress)[47]. The statistically significant changes observed in the content of irCRF were: increases in the LC by acute and chronic stress and in the periventricular nucleus and anterior hypothalamic area by chronic stress; and decreases in the median eminence-arcuate nucleus area by acute and chronic stress, in the medial preoptic area by acute stress and in the dorsal vagal complex by chronic stress. The decreases following acute stress presumably indicate increased release (and hence loss) of CRF from those areas, but the increases are difficult to interpret. After chronic stress, decreases may still reflect enduring release, while increases may reflect increased synthesis to compensate for the increased release. Apparently, prolonged stress is necessary to produce such effects, because Deutch et al. found no statistically significant changes in irCRF concentrations in several dopamine-rich brain areas after 20 min of footshock (0.2 mA circa 1 per s)[65]. Nevertheless, Owens et al. found that a single dose of the anxiolytic drugs, alprazolam (1 mg/kg) or adinazolam (10 mg/kg) increased hypothalamic concentrations of CRF and decreased those in the locus coeruleus 1 h later[178], effects opposite to those observed following acute and chronic stress discussed above.

3.2. *CRF receptors*

Using CRF and CRF analogs, it has been demonstrated that the characteristics of cerebral CRF-binding sites are similar to those described for the pituitary. Binding of CRF or CRF analogs in both rat and primate brain was saturable, reversible and of a high affinity with a dissociation constant (K_D) in the nanomolar range[48,62].

CRF-binding in the pituitary stimulates the activation of adenylate cyclase and the accumulation of cyclic adenosine monophosphate (cAMP), an action thought to regulate the release of ACTH[89,260]. Similarly, binding of CRF in the brain stimulates activation of adenylate cyclase[2,48,244,260]. CRF stimulation of adenylate cyclase is regulated by guanine nucleotides and divalent cations, just as occurs with other receptors coupled to adenylate cyclase[48]. The potency of CRF analogs in activating brain adenylate cyclase correlated well with their potency in activating ACTH secretion from pituitary cells and the adenylate cyclase activation by CRF in brain tissue was blocked by the CRF-antagonist, α-helical CRF_{9-41} (ahCRF)[48]. Nevertheless, Chen et al. found that the correlation between CRF-stimulated adenylate cyclase and binding sites in various brain regions was poor[48].

The regulation of cerebral CRF-binding sites appears to be different from that in the pituitary. The number of pituitary CRF-binding sites progressively decreases following adrenalectomy, accompanied by a decreased activation of adenylate cyclase[262,263]. Interestingly, although the number of binding sites and cyclase activation were decreased by adrenalectomy, there was a 3-fold *increase* in CRF-stimulated ACTH release in vitro[263]. Consistent with the effects of adrenalectomy, the number of binding sites was increased by corticosterone[108] or immobilization stress[109]. Chronic infusion of CRF itself desensitized the pituitary CRF receptor-adenylate complex; there were decreased numbers of binding sites, decreased activation of adenylate cyclase and decreased ACTH secretion in response to CRF[264]. However, neither the number of binding sites nor the coupling to adenylate cyclase in the brain was significantly altered by any of these treatments[108,109,262,263].

Although adrenalectomy has no effect on the number of CRF-binding sites or CRF-stimulated adenylate cyclase activity in the brain, alterations in the number of brain CRF-binding sites have been observed under certain conditions. In Alzheimer's disease victims, there was a decrease in irCRF in frontal, temporal and occipital cortices[21,64] that was accompanied by an increase in the number of binding sites[64]. Consistent with the cholinergic deficit in Alzheimer's disease, chronic atropine treatment of rats resulted in an increase in CRF-binding sites in the cerebral cortex[60]. Chronic treatment with the antidepressants, imipramine or desmethylimipramine did not alter CRF binding in many regions of brain, except in the brain stem, where imipramine increased binding[96]. Chronic treatment with diazepam, alprazolam or adinazolam (10 mg/kg daily for 28 days) resulted in decreased CRF-binding in the frontal cortex and hippocampus[96]. Chronic treatment with cocaine (20 mg/kg for 15 days) decreased CRF-binding in medial prefrontal cortex, nucleus accumbens, olfactory tubercle, frontal cortex and amygdala, and increased it in the substantia nigra and ventral tegmental area (VTA)[91]. Intracerebral 6-hydroxydopamine treatment (combined with desmethylimipramine to confine damage to dopaminergic cells) increased CRF-binding in most regions containing dopaminergic neurons, including medial prefrontal cortex, caudate, globus pallidus, anterior hypothalamus, medial forebrain bundle, substantia nigra and VTA[91]. Thus 6-hydroxydopamine treatment prevented almost all the effects of cocaine[91]. These results indicate that the CRF system is a dynamic one, capable of making compensatory changes to perturbations in normal function.

3.3. Neurochemical responses to administered CRF

One recent study examined changes in glucose utilization with the 2-deoxyglucose procedure 10 min after i.c.v. administration of CRF[210]. Changes were observed in a large number of brain areas, including locus coeruleus and the median raphe nucleus. However, the dose of CRF used was so high (more than 30 μg) that it is difficult to interpret these changes in physiological terms. Responses of the various neurotransmitter systems to the administration of CRF will be discussed below.

4. PHYSIOLOGICAL EFFECTS OF ADMINISTERED CRF

4.1. Endocrine effects of administered CRF

CRF is generally considered to be the primary activator of the HPA axis. Although other factors have been shown to possess corticotropin-releasing activity, the CRF of Vale et al.[246] appears to be the most potent[187]. Moreover, studies with specific CRF antagonists indicate that CRF is the major factor causing elevations of plasma ACTH during stress. Rivier et al. initially demonstrated that immunoneutralization of endogenous CRF by systemic administration of CRF antiserum could prevent the elevation of plasma ACTH in rats exposed to ether[193]. This result was replicated by Ono et al.[177] and Nakane et al.[164] and others have shown effective immunoneutralization following cold-water swims, immobilization or trauma due to bone fracture[164] and formalin or brief restraint[150]. Curiously, Ono et al. reported that antisera to CRF administered i.c.v. attenuated elevations of plasma ACTH[177]. Although i.c.v. antiserum was less effective than i.v., it is not clear why i.c.v. antisera had any effect at all. Subsequently, Rivier et al. reported reversal of ether-induced elevations of plasma ACTH with peripheral administration of the antagonist, ahCRF (1 mg i.v.)[194]. In unstressed rats, Conte-Devolx et al.[54] showed that antibody to CRF decreased plasma concentrations of ACTH and β-endorphin, but not α-melanocyte-stimulating hormone (α-MSH), suggesting that β-endorphin, but not α-MSH is co-released with ACTH by CRF.

VP has a powerful synergistic action on CRF-induced ACTH secretion[8,194]. VP may have some intrinsic ACTH-releasing activity[150,194], but its ability to elevate plasma ACTH in conscious animals is largely dependent on the presence of CRF[187,192]. The role is illustrated by the study of Linton et al.[150] mentioned above, who found that immunoneutralization of VP decreased the effects of formalin or restraint on the elevations of plasma ACTH, but to a lesser extent than CRF antiserum. However, VP antiserum complemented the effects of CRF antiserum.

Because the anterior pituitary is outside the blood–brain barrier, the HPA is best activated by systemic administration of CRF. However, CRF administered i.c.v. also activates the HPA axis in rats (0.3–1.0 μg)[175,254], mice (1 μg)[69] and monkeys (0.8–80 μg)[116,121,196]. It is not clear whether this occurs because of leakage of the CRF from the cerebral ventricles to the periphery or because of a direct action within the CNS. In one study, a high dose of CRF (10 μg) i.v. elevated plasma corticosterone in dexamethasone-treated rats[61], suggesting that there may be a direct adrenal effect of CRF at this high dose. However, according to Ono et al.[176] there may be a positive ultrashort feedback loop for CRF such that intracerebral CRF stimulates its own release. If this is the case, i.c.v.-administered CRF would stimulate the release of endogenous CRF. This mechanism would explain the HPA activation following i.c.v. CRF. Because i.c.v.-administered CRF might not reach all the relevant sites in the brain, an activation of endogenous CRF systems could account for the potency of i.c.v.-administered CRF. However, negative feedback of CRF on its own release has been suggested by in vitro studies[45], although other studies have been inconclusive[187].

I.c.v. CRF administration (0.5–10 μg) inhibits the secretion of luteinizing hormone (LH)[175,188,241] and growth hormone (GH)[127,175,189], but not follicle-stimulating hormone (FSH)[188,241], thyroid-stimulating hormone[175] or prolactin (Prl)[177,241]. Prolonged i.c.v. CRF administration in rats decreased LH and testosterone secretion in male rats[159]. I.v. administration of a single dose of CRF (10–100 μg) had no effects on LH[188] or GH secretion[127,189] in rats. However, in ovariectomized rhesus monkeys[174] and women[13], intravenous infusion of CRF inhibited LH and FSH secretion. Moreover, chronic i.v. administration of CRF (5 μg/day) to rats decreased LH, but did not alter testosterone or Prl secretion[190]. The chronic effect on LH appeared to be mediated by adrenal steroids, because it was mimicked by ACTH administration and the effect of ACTH was absent in adrenalectomized animals[190].

By contrast with the lack of effect of i.c.v. CRF, i.v. CRF increased Prl secretion in ovariectomized rhesus monkeys (100 μg)[253] and rats (10 μg)[162]. The effect in monkeys was blocked by prior administration of naloxone, suggesting a role for endogenous opiates.

The effect of i.c.v. CRF on LH secretion in rats appears to be a central one, because CRF inhibited gonadotropin (GnRH) secretion from hypothalamic slices in vitro[87,170], and GnRH secretion into the portal blood in vivo[183]. Endogenous opiates may also be involved because naloxone[6] or antiserum to β-endorphin[182] attenuated the effect of i.c.v. CRF on LH secretion in rats. Naloxone also reversed the effect of

peripherally administered CRF on LH and FSH secretion in rhesus monkeys[90] and women[13]. An involvement of endogenous opioids in GnRH secretion is consistent with the data on the effects of CRF on sexual behavior (see Ref. 218 below). The effect of CRF on GH secretion appears to involve an inhibition of somatostatin (SS) secretion, because peripheral administration of antisera to SS prevented this effect of i.c.v. CRF (10 μg)[127,191]. However, CRF has been reported to stimulate SS release from fragments of median eminence in vitro[1].

CRF has minor effects on insulin and glucagon secretion. According to one report, plasma concentrations of insulin were slightly reduced and those of glucagon slightly increased 2 min after i.v. CRF, whereas the reverse changes were observed 10 min after CRF[126]. These effects of CRF may be exerted on pancreatic cells, but the results have been conflicting (see Ref. 126). CRF has also been reported to inhibit the secretion of TRH from rat hypothalamus in vitro[160].

Each of the above-mentioned changes in hormone secretion is commonly observed in stress[72,154]. A physiological role for CRF in mediating these stress-induced changes in hormone secretion is suggested because various CRF antagonists can reverse or attenuate the effects of stressors. Electric shock-induced decreases in GH[191] and LH secretion in male rats[195] were prevented by the administration of ahCRF (100 μg i.c.v.). These effects were apparently central because 500 μg of ahCRF administered i.v. had no significant effects[177,195]. Ono et al.[177] showed that i.c.v. (but not i.v.) administration of antibody to CRF (3 μl) prevented the ether-induced decrease in GH secretion, along with the suppression of the elevation of plasma ACTH. In this study, CRF antisera did not alter basal LH secretion or the stress-induced changes. I.c.v. antiserum to CRF did not reverse the stress-related elevations of plasma Prl[177].

4.2. Autonomic effects of administered CRF

I.c.v. CRF increases the plasma concentrations of norepinephrine (NE) and epinephrine (EPI) in both rats (35 μg)[37] and dogs (120 μg)[35]. Accompanying these changes in circulating catecholamines were increases in plasma glucose and glucagon and bodily oxygen consumption[37,38] and mean arterial pressure (MAP) and heart rate[84]. Grosskreutz and Brody[97] found i.c.v. CRF (0.15–3.4 μg) to increase heart rate and vascular resistance in non-muscular vascular beds, both of which could be caused by sympathetic activation. In dogs, plasma VP was also elevated by 120 μg CRF i.c.v.[35]. An adrenomedullary involvement in these changes is indicated because i.c.v. CRF (0.4–40 μg) also increased the electrophysiological activity of the splanchnic (adrenal) nerve[137]. However, the sympathetic nervous system is probably activated also, because the autonomic ganglionic blocker, chlorisondamine, prevented the CRF-induced (10 μg i.c.v.) increases in plasma glucose, NE and EPI[38] and attenuated or reversed those in MAP and heart rate[84,85]. The effects of CRF are almost certainly centrally mediated, because hypophysectomy or adrenalectomy did not alter the elevations of plasma glucose[38], nor did these treatments or dexamethasone pretreatment alter the effects on MAP and heart rate[85]. Moreover, i.v. CRF (35 μg) *decreased* MAP, apparently with a reflex tachycardia[85] and peripheral administration of antibody to CRF which blocked the elevation of plasma ACTH by i.c.v. CRF failed to prevent the elevations of NE and EPI[36].

To investigate the intracerebral sites of action of CRF, Brown[34] injected CRF (1 μg) into 50 different brain sites. Although some sites were unresponsive to CRF, none of the sites showed elevations of plasma NE greater than those observed from third ventricle injections.

A physiological role for CRF in activating the sympathetic nervous system is suggested by studies with CRF-antagonists. I.c.v. ahCRF (100 μg) did not alter basal concentrations of NE or EPI[40], but reversed the i.c.v. CRF-induced elevations of plasma catecholamines[39]. The same dose of ahCRF reversed the ether-induced increase in plasma EPI, but not that of NE[39]. This could be interpreted to mean that the activation of the sympathetic nervous system is more sensitive than the adrenal medulla to the blockade of central CRF-receptors.

Interestingly, Fisher has recently provided evidence that i.c.v. CRF (1 or 10 μg) modifies baroreflex control of heart rate and that this effect is prevented by blockade of the vagus (with atropinemethylnitrate), but not of sympathetic output (with propranolol)[83]. Thus i.c.v. CRF may affect both the sympathetic and the parasympathetic nervous systems.

By contrast with the increased blood pressure caused by i.c.v. CRF, systemic CRF (10–35 μg) causes hypotension[85,130] and, according to one study, bradycardia[130]. The bradycardia, but not the hypotension, was prevented by hypophysectomy, dexamethasone or naloxone, but not by vagotomy. Thus Kiang and Wei[130] speculated that the bradycardia was primarily due to secretion of opioid peptides from the pituitary, whereas the hypotension was largely due to dilation of the mesenteric circulation, increasing blood flow to the gut.

CRF has also been implicated in the pyrogenic activity of interleukin-1 (IL-1). IL-1 injected either peripherally or i.c.v. elevates the body temperature of rats. This effect is apparently due to a sympathetic activation of metabolism in brown adipose tissue[58]. Intriguingly, i.c.v. ahCRF (25 μg) reversed the effect of human recombinant

IL-1β (50 ng i.c.v.) on body temperature and resting oxygen consumption[199]. This suggests that the thermogenic effects of IL-1β are exerted by release of brain CRF, which in turn activates the sympathetic nervous system to increase brown adipose tissue metabolism. Surprisingly, the pyrogenic effect of IL-1α was not reversed by ahCRF and, unlike that of IL-1β, appeared to be sensitive to prostaglandin synthesis inhibitors[43].

4.3. Electrophysiological effects of administered CRF

Eberly et al.[76] reported regionally specific responses to iontophoretically applied CRF. Inhibition of cell firing was recorded in the thalamus and lateral septum, whereas excitation occurred in the cortex and hypothalamus. I.c.v. CRF also affected the electrographic activity of rats[77,153,259]. In the study by Ehler et al.[77] low doses (0.01–0.1 μg) produced behavioral arousal accompanied by activation of the EEG characteristic of arousal. Higher doses (1–25 μg) also produced electrographic symptoms of arousal, but after a delay of 1–3 h seizure activity occurred. Marrosu et al.[153] compared the activity of rat and ovine CRF. Both activated the EEG at doses of 0.1 or 1.0 μg and caused spiking at 10 μg, but the latter effect was confined to the hippocampus with rat but not with oCRF. None of these effects of CRF were altered by naloxone. I.c.v. administration of CRF (0.01–0.1 μg) produced decreases in slow-wave sleep[78].

The effects of i.c.v. CRF on the spontaneous and sensory-evoked activity of LC neurons has been examined in rats. In anesthetized animals, i.c.v. CRF increased the spontaneous discharge rate of LC neurons. This effect was statistically significant at doses of 1 and 3 μg, but not 0.3 μg[248,250]. Similar, but less consistent effects were observed with direct administration of CRF into the LC. I.c.v. CRF decreased the excitatory component and increased the inhibitory component of the sensory-elicited response of LC neurons[248]. The overall effect of CRF on the sensory-elicited response of these cells was to disrupt the pattern of response and to decrease the signal-to-noise ratio. In unanesthetized rats, i.c.v. CRF also increased the spontaneous discharge rate[249], at doses of 1 or 3 μg, but not 0.3 μg. Although CRF did not significantly affect either the excitatory or inhibitory responses to sensory stimuli, the overall effect was to decrease the signal-to-noise ratio. These electrophysiological data complement the neurochemical findings of increased production of NE catabolites following intracerebral CRF administration (see below). Because the LC has been postulated to regulate arousal or vigilance states, the ability of CRF to increase the spontaneous activity of LC neurons suggests that CRF might act to increase arousal or vigilance[250]. The behavioral significance of the ability of CRF to disrupt neuronal responses in the LC to sensory stimuli is presently unclear.

That endogenous CRF might act to regulate the increase in LC discharge rate observed during stress is suggested by the ability of ahCRF (50 μg) to block the nitroprusside-induced increase in LC firing[247,251].

In hippocampal slices, high concentrations of CRF (more than 0.25 μM) usually depolarized both CA1 and CA3 pyramidal neurons, accompanied by increases in the spontaneous firing rate[5,213]. At lower concentrations (0.01–0.2 μM) CRF also reduced the magnitude and duration of afterhyperpolarizations after spontaneous or current-induced outbursts of action potentials. The enhanced discharge activity of hippocampal neurons is consistent with the CRF-induced changes in electrographic activity recorded in vivo described above.

4.4. Gastrointestinal effects of administered CRF

CRF administration has been shown to have widespread effects on gastrointestinal function. The effects of CRF include a whole spectrum of activities known to be affected in stress, including gastric acid secretion, bowel emptying and a variety of measures of gastrointestinal motility. The effects are complex and appear to depend upon the species and to some extent on the form of the stress. Generally, stress or CRF inhibit gastric acid secretion and gastric emptying, while stimulating large bowel transit and fecal excretion.

4.4.1. Gastric acid secretion. Taché et al.[236] first showed that intracisternal (IC) CRF (1.5, 5 or 15 μg) inhibited gastric acid secretion in rats. A direct central effect of CRF was suggested because its effect was present in hypophysectomized animals and local application of CRF (1.5 μg) into the lateral hypothalamus, but not the dorsomedial frontal cortex was effective. The effect of IC CRF was prevented by vagotomy, yohimbine treatment or adrenalectomy. In subsequent work, the effect of CRF (1–4 μg) was localized to the PVN or ventromedial hypothalamus[103]. Similar effects were found with lateral ventricle injection of CRF (0.6 or 12 μg)[66]. In these studies, the effects of i.c.v. CRF were reversed by chlorisondamine, bretylium, adrenalectomy or i.c.v. ahCRF (50 or 100 μg), but not by vagotomy or naloxone[66].

Lenz et al.[141,142] observed a similar decrease of gastric acid secretion with third ventricle application of CRF (0.6–36 μg/kg) in dogs. As in rats, the effect was reversed by chlorisondamine, but only partially by naloxone or a VP antagonist and not affected by vagotomy. These effects of i.c.v. CRF can best be explained by activation of the sympathetic nervous system which occurs in parallel with these effects at these doses of CRF[142].

I.v. CRF also inhibited gastric acid secretion in rats

(25–150 μg/kg)[144,237] and anesthetized and unanesthetized dogs (25–100 and 6–60 μg/kg, respectively)[131]. This effect in rats was not altered by naloxone (5 mg/kg) or indomethacin (10 mg/kg) pretreatment, nor by adrenalectomy or hypophysectomy, but was partially prevented by vagotomy[237].

A role for endogenous CRF in the effects of stress on gastric acid secretion is suggested, because two groups have observed reversal of stress-related effects by ahCRF. Stephens et al.[227] have shown that IC ahCRF (10 or 50 μg) administered to rats reversed the surgery-related decrease and Lenz et al.[144] found ahCRF (5–50 μg) i.c.v., but not i.v., reversed the partial restraint-induced inhibition of gastric acid secretion.

4.4.2. Gastric emptying. In rats, i.c.v. (0.6–10 μg)[143,258] or IC CRF (0.3–1 μg)[238] decreased gastric emptying of saline. Taché's group found that the effects of IC CRF were not prevented by naloxone treatment, nor by adrenalectomy, but were reversed by vagotomy. Moreover, i.v. administration of antiserum to CRF did not prevent the effect of IC CRF administration[238]. However, Lenz et al.[143] found that the effects of lateral ventricle infusion of CRF (0.6 or 6 μg) were completely abolished by chlorisondamine, naloxone or bretylium treatment, but not by adrenalectomy, hypophysectomy or truncal vagotomy, suggesting the involvement of the sympathetic nervous system. Surgical stress or partial restraint stress also inhibited gastric emptying and these effects were reversed by i.c.v. ahCRF (60 μg)[144,239].

I.v. CRF (0.15–10 μg)[144,238,258] or i.p. (6 μg)[143] also inhibited gastric emptying in rats. This effect of CRF was prevented by i.v. antiserum to CRF[238], but not altered by bretylium or chlorisondamine[143].

However, in the mouse, gastric emptying of a non-nutritive meal was stimulated by i.c.v. CRF (0.15 μg)[41,100], but not by the same dose of CRF administered IP nor by corticosterone (300 ng) or ACTH (375 μU)[41]. The effects of i.c.v. CRF resembled those of exposure to 20 min acoustic or cold stress[41,100]. The effects of acoustic stress were reversed by i.c.v. ahCRF (200 ng) or antiserum to CRF[98]. Curiously, the effects of acoustic or cold stress or i.c.v. CRF were reversed by antiserum to CRF-administered IP[41].

In the dog, gastric emptying of a non-nutritive meal was inhibited by i.v. CRF (0.6–6 μg/kg/h)[131,179,180]. I.c.v. CRF (0.7–3.0 μg/kg) had no such effect[180]. The effects of i.v. CRF were not altered by naloxone[179] or propranolol[180].

4.4.3. Gastrointestinal motility. I.c.v. CRF (0.3–6.0 μg) inhibited small bowel transit and markedly increased large bowel transit in the rat[143,144,260]. These effects were reversed by chlorisondamine or vagotomy, but not by bretylium[143]. Whereas the effect on the small bowel was reversed by naloxone, the large bowel effect was not[143]. Partial body restraint mimicked these effects of i.c.v. CRF[144,258]. The effects of both CRF and partial restraint were reversed or attenuated by i.c.v. ahCRF (50 μg)[144]. The antagonist was ineffective against restraint or i.c.v. CRF when given i.v.[144]. In the study of Williams et al.[260] CRF (0.3 μg) or partial restraint stress also increased fecal excretion. The effect of the restraint was reversed by ahCRF (50 μg i.c.v.). Moreover, i.v. or i.c.v. ahCRF (50 μg) reversed the effects of partial restraint on small and large bowel transit and on fecal excretion.

I.v. (1–10 μg)[260] or IP CRF (6 μg)[144] stimulated large bowel transit, but inhibited[260] or did not alter small bowel transit[144]. These effects were not altered by bretylium or chlorisondamine[144].

Migrating myoelectric complexes (MMCs) can be recorded by cutaneous electrodes on the abdomen. They are the basic motor pattern of stomach and small intestine, characterizing the fasted state. The activity is cyclic (90–120 min) and is involved in mixing the contents of the bowel and in movement of matter and nutrient absorption in the upper gut. Their occurrence in fasted dogs was inhibited by 60 min acoustic stress[99]. I.c.v. CRF (0.02–0.1 μg/kg) suppressed gastric MMCs for 4–5 h[42,99]. I.v. CRF (0.1 μg/kg) had no such effect[42]. The effect of i.c.v. CRF was abolished by thoracic vagotomy[99].

In fed sheep, CRF decreased antral activity[200]. IC CRF (0.1–1 μg) decreased 2-deoxyglucose- or TRH-stimulated gastric contractility (the antral motor response) in the rat[88]. I.v. CRF was also effective, but approximately 10 times less potent.

4.4.4. Gastric ulceration. Intracisternal administration of CRF (5–10 μg) did not elicit gastric lesions[95,135,165]. However, intrahypothalamic injection of CRF (2 μg per side) or 5 μg i.c.v. prevented the lesions caused by 1–4 h cold restraint[102,135]. An i.c.v. injection of 50 μg ahCRF reversed the effect of CRF, but not that of cold restraint[135]. These results suggest that CRF has an amelioratory effect on stress-induced gastric lesions.

Apparently, CRF can have both central and peripheral effects on the gastrointestinal function. Judged by the use of CRF antagonists, these effects are independent. CRF decreases gastric emptying, gastric acid secretion and small bowel transit, while increasing large bowel transit and fecal excretion. An exception is the mouse, in which gastric emptying of a non-nutritive meal was increased. However, in most cases the effects of CRF resemble those of various stressors. The effects of i.c.v. or IC CRF seem to be partly mediated by the sympathetic nervous system, with the possible participation of the vagus. On the other hand the effects of i.v. CRF seem to be independent of the sympathetic nervous system, but may depend upon an intact vagus.

5. BEHAVIORAL RESPONSES TO ADMINISTERED CRF

CRF administered to animals elicits a number of behavioral responses. In almost every case intracerebral administration appears to be necessary to elicit significant behavioral effects. Peripheral administration is either ineffective or much greater doses are necessary to produce effects, which may even differ from those elicited by intracerebral injections. Many of the behavioral responses to CRF resemble those observed during stress. Consistent with this, the effects of CRF are frequently opposite to those observed following administration of benzodiazepines and the latter have often been found to reverse the effects of CRF. Moreover, in some cases, CRF-antagonists have been found to reverse or attenuate stress-induced changes. In this section, we review the behavioral data reported following CRF administration and other relevant observations on those particular behaviors.

5.1. Locomotor activity

The effect of CRF on locomotor activity is dependent on both the dose of CRF and the testing conditions. In rats tested in a familiar environment, i.c.v. CRF produced a dose-dependent activation of locomotor activity at doses between 0.1 and 10 μg[27,44,211,230]. This effect of i.c.v. CRF was apparently independent of pituitary-adrenal activation because it was not blocked by either dexamethasone at a dose that prevents the increase in plasma corticosterone induced by CRF[27,32], nor by hypophysectomy[75]. Further, i.v. CRF (8 μg) did not alter locomotor activity when tested under similar conditions[27]. The CRF antagonist, ahCRF, blocked the CRF-induced increase in locomotion, suggesting that CRF affects this behavior through CRF receptors[32]. The benzodiazepine antagonist, Ro 15–1788, did not alter the effect of i.c.v. CRF on behavior in this paradigm[33]. CRF was 10 times more potent when injected directly into the locus coeruleus as compared to the cerebral aqueduct[44].

In an open field test, the behavioral responses of an animal in a relatively large and open novel environment are observed. In this test, low doses of i.c.v. CRF increased locomotor activity of rats (0.01 μg, but not 0.001 or 0.0001 μg)[230] or mice (0.2 μg)[140]. The increase in activity was primarily in the central region in both species. However, higher doses of CRF (1–2 μg) decreased activity[25,26,114,140,230]. When observed in a novel cage with wood shavings present, CRF (3 μg) increased horizontal locomotor activity but decreased the number of rears[211]. In mice, CRF injected into the hippocampus (0.01 μg each side) or amygdala (0.02 μg each side) increased locomotor activity in the center of an open field[139]. However, Rosenthal and Morley[197] found no effect of i.c.v. CRF (0.01–10 μg) in mice deprived of food during 18 h.

The increased activity observed at lower doses of CRF in an open field resembles that observed following exposure of animals to a stressor[139,140,198]. Further, the locomotor-activating effect of CRF in mice was reversed by pretreatment with 2 mg/kg diazepam, a dose that had no sedative effects when administered in the absence of CRF[140]. Thus, low doses of CRF have stress-like properties increasing locomotor activity in the open field.

5.2. Ingestive behavior

I.c.v. CRF inhibited feeding in food-deprived animals in both familiar and novel environments. At doses greater than 1 μg, i.c.v. CRF decreased food intake in food-deprived rats[9,94,134,163] and in mice[197]. The CRF-induced decrease in feeding was not altered in hypophysectomized animals (5 μg CRF)[163], nor by pretreatment with dexamethasone (0.5 μg CRF)[27]. CRF (5 or 10 μg, but not 1 μg) also inhibited muscimol-, norepinephrine- and dynorphin-induced[145] and ethylketocyclazocine-induced feeding (5 μg)[94]. The inhibitory effect of i.c.v. CRF on feeding is similar to that observed following restraint for 1 h[134]. Moreover, ahCRF (50 μg) -injected i.c.v. attenuated this effect of restraint[134], suggesting that endogenous CRF is involved in the restraint-induced decrease in feeding. Interestingly, interleukin-1 (1–25 μg/rat IP) induces anorexia in rats. CRF appears to be implicated in this effect, because i.c.v. antiserum to CRF (10 μl) prevented the effects of IL-1β (2 μg IP)[245].

In an open field in which a pellet of food was secured to the center (and presumably more aversive) region, CRF (0.5 and 1.0 μg, but not 0.1 μg) decreased both the number of approaches to the food and the average amount of food consumed per approach[25,26]. This effect was opposite to that observed following administration of benzodiazepines[24] suggesting that CRF enhances the anxiogenic nature of the novel environment. However, because similar doses of CRF inhibit feeding in a familiar environment, its ability to decrease feeding in an open field may be independent of effects on the animal's reaction to the novel environment.

Interestingly, CRF at a lower dose (0.1 μg) increased feeding in 24-h food-deprived animals[94]. The ability of CRF to exert opposite effects on the same behavior depending on dose resembles that observed with locomotor activity in the open field.

5.3. Antinociception

Human patients injected with CRF i.v. (1 μg/kg) reported less postoperative pain (molar extraction) than those that received placebo[104]. Likewise, rats injected i.v. with oCRF (150 μg) showed almost as much antinoci-

sponsiveness as those that received 2.5 mg/kg morphine in the hot-plate test[104]. This effect is probably due to release of β-endorphin, because plasma concentrations were elevated by the oCRF infusion[104] and the antinociceptive effects were reversed by hypophysectomy or dexamethasone treatment[106]. Doses of CRF up to 3 μg i.c.v. failed to alter latencies of rats to respond in either the hot-plate or the tail-flick tests for analgesia[211].

CRF (120 μg/kg) administered SC decreased the hyperalgesia, edema and hyperthermia observed in the carrageenan model of inflammation[105]. These effects were blocked by adrenalectomy but not by hypophysectomy. CRF also had an antinociceptive effect when injected directly into the inflamed hindpaw (0.4 μg), but the local injections had no effect on measures of inflammation.

5.4. Grooming

I.c.v. CRF increased grooming in rats at doses above 0.3 μg (0.3–20 μg) when tested in either the open field or familiar testing chambers[25,26,27,74,114,163,207,211,212,254]. Doses below 0.3 μg were ineffective in most of these studies[25,74,163,254], although 0.1 μg was effective when rats were tested in a shock chamber[212] or in the social interaction test[70]. Thus, the threshold for CRF to induce grooming may depend on the testing environment.

The CRF-induced increase in grooming is not dependent on the pituitary-adrenal axis because this effect was not inhibited by hypophysectomy[163] or dexamethasone pretreatment[27,74]. Nor did SC administration of CRF increase grooming behavior in rats[27,211]. CRF-induced grooming was prevented by pretreatment with naloxone[74]. Although CRF increases grooming in rats, very little response was observed in mice injected with up to 1 μg i.c.v.[74]. In fact, one report found decreases in mice that had been briefly anesthetized before testing with 0.01–10 μg CRF i.c.v.[196]. It is unclear whether this represents a difference between the species in the ability to respond to the peptide or in the sensitivity of the mice to CRF.

Effects on grooming may be related to stress, because rats exposed to a novel environment display increased grooming behavior that habituates with repeated exposure to the same environment[49] and is attenuated by benzodiazepine pretreatment[73].

5.5. Conflict tests and other tests of anxiety

The effect of CRF has been examined in a number of tests commonly used in the study of anxiety and anti-anxiety drugs. These tests include the Geller–Seifter conflict test, the social interaction test and the acoustic startle response. As described above, CRF decreased the number of approaches and the amount of food eaten in an open field, an effect opposite to that observed following benzodiazepine administration[25,26].

5.5.1. Geller–Seifter conflict test. In the Geller–Seifter conflict test, anxiolytic agents increase behavioral responding (lever press for food) in the presence of footshock (i.e. punished responding), without effects on responding in the absence of the aversive stimulus (unpunished responding). In this test, i.c.v. CRF (0.5 or 1.0 μg) decreased both punished and unpunished responding[30,33]. Dexamethasone pretreatment did not inhibit the effect of CRF in this test[32]. AhCRF blocked this effect of CRF, suggesting that CRF acts through specific receptors[31]. Chlordiazepoxide (CDP: 5 mg/kg) completely antagonized the effect of CRF on the conflict component and partially reversed the CRF-induced decrease in the unpunished component[30,33]. At this dose, CDP had no effect on unpunished responding in the absence of CRF, but did increase punished responding. The benzodiazepine antagonist, Ro 15–1788, also antagonized the inhibitory effect of CRF on punished responding at a dose that did not affect punished responding in the absence of CRF[33]. This may reflect some partial agonist properties of Ro 15–1788. By contrast, a benzodiazepine inverse agonist, FG 7142, decreased punished responding and potentiated the effects of CRF in this paradigm[33].

These results are consistent with CRF having a stress-like or anxiogenic effect. The simple explanation that CRF enhances the sensitivity of the animal to the pain of the electric shock used in this test is unlikely because CRF did not increase the animal's sensitivity to noxious stimuli in the hot plate and tail flick tests[30,211,212]. In fact, as mentioned above i.v. CRF caused apparent analgesia in rats in the hot plate test[104]. However, the fact that unpunished responding was also depressed suggests that CRF may have produced a generalized inhibition of responding. The effects of CRF could also be related to its inhibitory effect on feeding, observed at similar doses (see above). However, the reversal of the effect of CRF on punished responding by doses of CDP or Ro 15–1788 that independently had no effect on this behavior, suggests that CRF may act on anxiogenic mechanisms.

In pigeons i.c.v. CRF (3.0–30 μg) decreased responding in a shock-motivated operant task[14]. This effect of CRF was antagonized by 10 or 30 μg/kg ahCRF i.c.v.

5.5.2. Social interaction. The social interaction test measures the amount of time that two animals spend in active contact with each other in an open field. Benzodiazepines increase, whereas benzodiazepine-inverse agonists decrease social interaction without affecting locomotor activity[81]. I.c.v. CRF (0.1 and 0.3 μg) decreased the time spent in social interaction in a familiar environment without affecting locomotor activity. This

effect of CRF was reversed by pretreatment with 5 mg/kg CDP[70].

5.5.3. Acoustic startle. The acoustic startle test measures skeletal musculature contraction in response to a sudden and intense acoustic stimulus. This response is increased under conditions associated with stress or anxiety[50]. I.c.v. CRF at 1 μg, but not 0.1 or 10 μg, significantly increased the mean startle amplitude[233]. This effect of CRF was antagonized dose-dependently by CDP (2.5–10 mg/kg)[233] and by ahCRF (1–25 μg i.c.v.)[234]. The actions of CDP and ahCRF were not related to non-specific depressant effects, because they did not inhibit the amphetamine- (CDP)[233] or strychnine-induced (CDP or ahCRF)[233,234] enhancements of the acoustic startle response. I.c.v. ahCRF (5 or 25 μg) dose-dependently reversed the effects of conditioned fear on acoustic startle[234]. Amygdaloid lesions prevented this effect of CRF[148].

5.5.4. Elevated plus maze. The elevated plus maze is another standard test to assess the anxiolytic/anxiogenic properties of drugs. In this task, File et al. found i.c.v. CRF (0.1 μg) decreased the time spent on the open arms, suggesting an anxiogenic effect. This effect that was not altered by Ro 15–1788 pretreatment[82].

5.5.5. Defensive withdrawal. Takahashi et al.[240] have used an open field modified by including a metal cylinder into which a rat can retreat. Behavioral testing starts with the rat inside the cylinder and measures of anxiety include the time taken to emerge from the cylinder, the total number of emergences and the mean time spent in the cylinder. AhCRF (50 μg i.c.v.) significantly decreased the time taken for a rat to emerge from the cylinder for the first time and also decreased the proportion of time the rat spent in the cylinder. When the rats had been familiarized with the apparatus on the previous day, i.c.v. CRF (0.3 μg) had an anxiogenic effect, increasing the latency to emerge from the cylinder and increasing the proportion of time spent in the cylinder. Peripheral administration of CRF (0.3 μg IP) was ineffective. The anxiogenic effects of intracerebral CRF in this task have been replicated by Butler et al.[44] (0.1 μg into the LC or 1 μg into the aqueduct) and Yang and Dunn (0.02–0.1 μg i.c.v.)[266]

5.5.6. Porsolt swim test. I.c.v. CRF (0.5 μg) decreased floating time in the Porsolt swim test, indicative of behavioral activation and perhaps anxiety[44]. The CRF was much more effective when injected directly into the locus coeruleus (0.01 μg).

5.6. Conditioned emotional responses

In a conditioned suppression paradigm, CRF (0.5 μg, i.c.v.) decreased responding to the conditioned stimulus (CS), suggesting that CRF increased the anxiogenic character of the test[50]. Although i.c.v. CRF decreased responding in the pre-CS component, the effect was greater in the CS component than in the pre-CS component. Evidence for a role of endogenous CRF in this behavior was obtained from studies with ahCRF, which at i.c.v. doses of 1, 5 and 25 μg significantly attenuated the CER[52]. These results are consistent with the hypothesis that CRF enhances anxiety.

5.7. Exploratory behavior

The effect of CRF on exploratory behavior in a complex novel environment has also been examined in mice and rats. The testing chamber used was a multi-compartment chamber (MCC) consisting of 9 interconnecting compartments within each of which a wire-mesh sphere was recessed in a hole in the floor, slightly below it[10,16]. Exposure of rats to restraint or repeated tail-pinch immediately prior to testing or white noise stress during testing decreased the time spent investigating the wire stimuli without affecting measures of locomotor activity[11]. Very similar results were obtained with prior restraint in mice[16]. This effect was reversed by a dose of naloxone that independently had no effect on this behavior[11,16]. I.c.v. CRF administered to mice (0.005–0.150 μg)[16,19] or rats (0.02–0.05 μg)[226] produced a stress-like response; the investigatory behavior was decreased in the absence of effects on locomotor behavior. The CRF-induced decrease in stimulus interaction time was also blocked by naloxone pretreatment at a dose (0.7 mg/kg) that had no effect in the absence of CRF[16]. The effect of CRF on exploratory behavior was also observed in hypophysectomized mice[20]. I.c.v. injection of ahCRF (10–50 μg), reversed the restraint-induced decrease in stimulus interaction[17]. These results suggest that restraint decreases exploratory behavior by a mechanism that involves the release of endogenous CRF. It is noteworthy that CRF administration also decreased exploratory behavior in primates (see below Ref. 120).

5.8. Shock-induced behaviors

5.8.1. Shock-induced freezing. Rats exposed to a brief footshock display an increase in freezing behavior characterized by a complete lack of movement and a crouched posture[23]. I.c.v. CRF (0.3 μg) had a biphasic effect on this response[212]. It increased the amount of freezing immediately following the shock, whereas at later times (16–20 min), CRF facilitated recovery. Because CRF increased grooming at these later times, it was postulated that the enhanced recovery from the shock-induced freezing was related to the increased grooming activity. Interestingly, CRF did not affect freezing behavior observed following a 60 s exposure to a hot-plate. However, there were a number of differences in the

TABLE I

Responses to intracerebrally administered CRF

Summary of responses to intracerebral administration of CRF. Injections were i.c.v. or intracisternal, except where otherwise indicated. * Injections into the amygdala, hippocampus, or hypothalamus. + Indicates an increase in the parameter, – a decrease (an anxiogenic effect for the Geller–Seifter test and the Porsolt swim test).

Measure	*Species*	*Effective dose of CRF (μg)*	*Effect of CRF*	*Effect of stress*	*Refs.*
Endocrine					
Plasma ACTH	rat	0.5–10	+	+	175
	monkey	180 μg/kg	+	+	121
Plasma corticosteroids	rat	0.1–1.0	+	+	44, 254
	mouse	1.0	+	+	69
	monkey	0.1–60 μg/kg	+	+	116, 121, 196
Plasma GH	rat	0.5–10	–	–	127, 175, 189
Plasma LH	rat	1–10	–	–	175, 188, 241
Plasma VP	dog	120	+	+	35
Plasma glucagon	rat	35	+		38
Physiological					
Plasma NE, EPI	rat	1–35	+	+	34, 37
	dog	120	+	+	35
	monkey	60 μg/kg	+	+	121
Plasma glucose	rat	35	+	+	37, 38
Arterial pressure	rat	10	+	+	84, 85
Heart rate	rat	10	+	+	84, 85
EEG	rat	0.01–1.0	+		77, 153
		1–25	seizures		77, 153
Locus coeruleus firing	rat	1–3	+	+	248, 250
Gastrointestinal function:					
Gastric acid secretion	rat	0.6–15	–	–	66, 103, 236
	dog	0.6–36	–	–	141, 142
Gastric emptying	rat	0.6–10	–	–	143, 144, 238, 260
	mouse	0.2–1.0	+	+	41, 100
Gastric ulceration	rat	4–10	–	+	95, 135, 165
Small bowel transit	rat	0.3–6	–	–	143, 144, 260
Large bowel transit	rat	0.3–6	+	+	143, 144, 260
MMCs	dog	0.02–0.1 μg/kg	–	–	42, 99
Fecal excretion	rat	0.3	+	+	260
Neurochemical					
DA release	rat	1–10	+	+	156
		20	+	+	125
	mouse	0.2–1.0	+	+	69
	pigeon	5.6–30	+		14
NE release	rat	1–10	+	+	44, 156
	mouse	0.2–1.0	+	+	69
	pigeon	5.6–30	+		14
5-HT release	pigeon	5.6–30	+		14
Behavioral					
Locomotor activity:					
Familiar environment	rat	0.1–10	+		27, 32, 44, 211, 230, 254
Novel environment	rat	0.2	+	+	198, 230
		>1.0	–	–	25, 26, 114, 211, 230, 254
	mouse	0.01–0.2*	+	+	139, 140
		2	–		140
Ingestion:					
Familiar environment	rat	0.1	+		94
		0.5–10	–	–	9, 94, 134, 145, 163
	mouse	0.05–10	–		197
Novel environment	rat	0.5–1.0	–		25, 26
Grooming	rat	0.1–20	+		25–27, 70, 74, 114, 163, 207, 211, 212, 254
	mouse	0.1–1.0	0		74
Geller–Seifter conflict test	rat	0.5–1.0	–		30, 33
Operant responding	pigeon	3–30	–		14
Social interaction	rat	0.1–0.3	–	–	70, 81
Acoustic startle	rat	0.5–1 (not 10)	+	+	59, 148, 233, 234
Elevated plus maze	rat	0.1	–	–	82
Defensive withdrawal	rat	0.02–0.3	+	+	44, 240, 265, 266
Porsolt swim test	rat	0.5	–	–	44
Conditioned emotional response	rat	0.5	+	+	50
Exploration – MCC	rat	0.02–0.1	–	–	11, 226
	mouse	0.005–0.15	–	–	16, 17, 19, 20
Shock-induced freezing	rat	0.3	+/– (biphasic)	–	23, 212
Shock-induced fighting	rat	0.01–0.5	+	+	242
Sexual behavior					
male	rat	2–4	–	–	152, 220
female	rat	0.5–2	–	–	152, 218, 219, 223
Passive avoidance	rat	0.00003–0.1	–		203, 254
		0.1*	+		138, 147

testing procedures between the footshock and hot-plate experiments, so that the significance of the different results obtained from these two paradigms is unclear. The shock-induced increase in freezing was partially antagonized by 25, but not 50 μg of ahCRF i.c.v.[122]. This protective effect of ahCRF was also observed when 20 μg was injected before re-exposure to the apparatus in which the rats had been shocked 24 h earlier[124].

5.8.2. Shock-induced fighting. Exposure of a pair of rats to intermittent footshock elicits boxing (upright posture without physical contact) and fighting behaviors. The frequency of each behavior is dependent upon various parameters including shock intensity. I.c.v. CRF (0.01 and 0.1 μg) increased boxing behavior at lower shock intensities (0.3 and 0.4 mA) and increased fighting behavior at 0.5 mA[242]. One μg CRF completely disrupted the behavioral response to 0.5 mA. The shock-induced fighting (at 0.6 mA) was reversed by 5 and 25 μg of ahCRF, injected i.c.v., suggesting the involvement of endogenous CRF in regulating this shock-induced fighting[242]. The minimum doses of CRF and ahCRF that affected behavior in this paradigm were similar to those affecting exploratory behavior in the MCC.

5.9. Sexual behavior

Normal reproductive function is inhibited in stress[152]. CRF (0.5–2 μg) inhibited lordosis behavior in ovariectomized, estrogen- or estrogen/progesterone-primed female rats when injected into the mesencephalic gray[218], arcuate-ventromedial area of the hypothalamus[223] and the medial preoptic area[219]. Injections of CRF into tissue sites outside these areas had no effect on lordosis behavior, indicating a site-specific effect of CRF on this behavior. In male rats, injections of CRF (2, 4 or 10 μg) into the third ventricle also impaired sexual behavior, with increases observed in the time to mount, the latency to ejaculate, the number of mounts without intromission and the number of intromissions before ejaculation[220]. In a sexually motivated learning task, Lee and Sung found biphasic effects of CRF injected into the amygdala[138]. Low doses (0.01 μg) enhanced retention, whereas higher doses (0.1 μg) inhibited it.

5.10. Conditioned avoidance responding

CRF had multiple effects in passive and active avoidance tests depending on the dose and route of administration[254]. In hypophysectomized rats, which display poor avoidance acquisition, 0.2 or 0.6 μg CRF given SC for 7 days increased acquisition. When tested in the pole jump shock avoidance test, 0.3 and 1.0 μg CRF SC facilitated extinction of the avoidance response in intact and adrenalectomized animals. Because the effect of ACTH or β-endorphin on extinction of the pole jump response is opposite to that of CRF[67], the involvement of the pituitary-adrenal axis in the effect of CRF is unlikely. CRF administered peripherally on postpartum days 1–5 improved acquisition of an active avoidance response at 35–37 days of age[112].

In the passive (inhibitory) avoidance test, SC administration of 0.03 μg CRF 1 h prior to testing improved performance 24 or 48 h later, whereas performance at 24 h was impaired by doses of 0.3 and 1.0 μg[254]. It is possible that the higher doses of CRF, which stimulated ACTH release, might have affected performance by increasing the concentration of circulating corticosteroids, which have been shown to impair performance in this task[22]. I.c.v. CRF (0.00003 and 0.0003 μg) injected either immediately following the training session or 1 h prior to testing, impaired performance 24 h after training[254]. Sahgal et al.[203] observed a similar impairment of passive avoidance performance by i.c.v. CRF (0.1 μg) when injected immediately post-training or 1 h prior to testing. When tested 48 h post-training 0.03 μg i.c.v. CRF injected 1 h prior to testing improved performance[254]. In contrast, 0.1 μg CRF injected into the amygdala immediately following training enhanced performance 24 h or 1 week later[147,138].

TABLE II

Effects of hypophysectomy, adrenalectomy, or dexamethasone treatment on the responses to intracerebral CRF administration

'+' indicates a CRF-induced increase in the measure; '–' a decrease. '0' indicates that the treatment does not alter the response to CRF; 'B' indicates that it blocks the response.

Measure	*CRF effect*	*Hypox effect*	*Adrex effect*	*Dex effect*	*Refs.*
Physiological					
Plasma glucose	+	0	0		38
Arterial pressure	+	0	0	0	85
Heart rate	+	0	0	0	85
Gastrointestinal function:					
Gastric acid secretion	–	0	B		236
			B		66
Gastric emptying	–	0	0		143
			0		238
Small bowel transit	–	0			143
Large bowel transit	+	0			143
Behavioral					
Locomotor activity	+	0		0	27,32
					75
Ingestion	–	0			163
				0	27
Grooming	+	0		0	163
				0	27
				0	74
Geller–Seifert test	–			0	32
Exploration (MCC)	–	0			20
Active avoidance	+	0	0		254

5.11. Primate behaviors

There have been relatively few studies of the behavioral effect of CRF in primates. When administered i.v. to chair-restrained rhesus monkeys, 10 and 125 μg/kg CRF increased struggling and investigatory behaviors[120]. When tested in their home cages, both doses increased vocalizations, threatening behavior and the time spent passively lying down. An increase in ingestive and self-directed behaviors was observed at the lower dose only, whereas decreases in environmental exploration, grooming and huddling behaviors were observed at the 125 μg/kg dose. I.c.v. CRF (20 and 180 μg) increased the general activity in chair-restrained rhesus monkeys, but did not significantly affect any specific behavior[121]. In the home cage, both doses of CRF increased vocalizations. At the higher dose an increase in huddling and lying down behavior was observed. CRF (10 μg) administered i.c.v. to infants separated from their mothers, inhibited behavior[123]. Lower doses did not produce observable changes. Although these behavioral changes were accompanied by increases in plasma CRF, ACTH and cortisol, they were not mimicked by peripheral CRF administration that resulted in higher circulating concentrations of all 3 hormones[123].

In squirrel monkeys, i.c.v. CRF had complex effects; generally, CRF increased measures of activation and vigilance[261]. CRF (10 μg) increased locomotor activity under several conditions, an effect that was prevented by ahCRF (10 μg i.c.v.). Vigilance (change of gaze) was enhanced by 0.1 or 1.0, but not by 10 μg CRF. Interestingly, 10 μg ahCRF had a similar effect, suggesting that this antagonist has partial agonist properties. The authors considered that the effects of the lower doses of CRF corresponded to increased vigilance, whereas higher doses increased escape and withdrawal[261].

6. CRF AND THE IMMUNE SYSTEM

CRF has been reported to have both direct and indirect effects on the immune system. In rats, i.c.v.

TABLE III

Effects of naloxone on the response to CRF

BEA means β-endorphin antiserum. B indicates that it blocks the effect of CRF; Att, attenuates the effect of CRF; 0, does not alter response to CRF; ?, the dose of CRF for this experiment was not explicitly stated in the report.

Measure	*CRF dose*	*Effect of naloxone*	*Effect of BEA*	*Effect of opiates*	*Refs.*
Endocrine					
LH secretion	10 μg	0			188
	10 μg		B		182
	10 μg	Att			6
LH and FSH secretion	100 μg/h IV	B			90 (monkey)
	100 μg/h IV	B			13 (woman)
Physiological					
Blood pressure increase	8 μg	B			207
Tachycardia	8 μg	B			207
Bradycardia	10 μg IV	B			130
Gastrointestinal function:					
Gastric acid secretion	10 μg?	0			236
	12 μg	0			66
	12 μg	Att			142
Gastric emptying	1 μg	0			238
	0.6 μg	B			143
Small bowel transit	6 μg	B			143
Large bowel transit	0.6 μg	0			143
Epileptic		B			153
Neurochemical					
DA release	1 μg	0			69
NE release	1 μg	0			69
Behavioral					
Locomotor	1 μg	0			132
	8 μg	B			207
Grooming	1 μg	B			74
	8 μg	B			207
Female sexual behavior	2 μg	B	B		218
(lordosis)	0.5 μg	Att	Att		219
Male sexual behaviors	4 μg	B			220
Exploratory behavior	0.05 μg	B			16

injection of CRF (0.1–1 μg) dose-dependently reduced splenic natural killer (NK) cell cytotoxicity measured 1 h after injection, with a minimum effective dose of 1 μg[117]. No such effect was observed with peripherally administered CRF (5, 10 or 20 μg/kg) nor with CRF (10^{-12}–10^{-6}) in vitro. The effect of i.c.v. CRF was antagonized by i.c.v. (100 μg) but not peripherally administered ahCRF (0.5 mg/kg). In a subsequent study, the decreased NK cytotoxicity induced by i.c.v. CRF (1 μg) was found to be parallelled by an increase in plasma NE and both effects were abolished by pretreatment (1 h) of the rats with chlorisondamine (3 mg/kg)[118]. However, the CRF-induced elevations of plasma ACTH and corticosterone were not altered by chlorisondamine, suggesting that the effect of i.c.v. CRF was mediated by the autonomic nervous system and not by ACTH or corticosterone. The depressant effect of repeated footshock treatment on NK cell activity was prevented by i.c.v. injection of antibody to CRF, suggesting that cerebral secretion of CRF mediates this effect of footshock[119].

Other workers have focussed on direct effects of CRF on lymphocytes. Webster and De Souza[255] found binding sites for CRF in mouse spleen, resembling those found in the pituitary. The binding sites were localized primarily in the red pulp and marginal zones and were not found in the periarteriole and peripheral follicular white pulp regions. The authors consider that this distribution suggests the absence of binding sites on B and T cells, consistent with their own observations of a lack of CRF binding in human peripheral blood lymphocytes and mouse thymus. The binding sites appear to be on splenic macrophages[256]. These results are partially consistent with those of Singh and Fudenberg who reported CRF binding sites on monocytes and to a lesser extent T cells from human donors[217].

McGillis et al.[157] reported that incubation of rat B lymphocytes with CRF (10^{-10}–10^{-7} M) in vitro stimulated proliferation. Interestingly, ahCRF was as effective as CRF. Consistent with this, CRF (10^{-10}–10^{-8} M) in vitro enhanced the ability of concanavalin A and phytohemagglutinin, but not pokeweed mitogen, to stimulate human lymphocyte proliferation and 10^{-9} M CRF increased

TABLE IV

Effects of CRF antagonists on responses in stress

B = blocked; 0 = no significant effect; Att = the effect was attenuated; ? = the dose was not specified.

Measure	*Stressor*	*ahCRF dose*	*ahCRF effect*	*CRF antibody*	*Refs.*
Endocrine					
Plasma ACTH	ether	1 mg IV	B	B	193, 194
	ether			B	177
	ether			B	164
	cold swims		B		164
	immobilization		B		134
	formalin			B	150
	restraint			B	150
Plasma GH	foot shock	100 μg	B		191
	ether			B	177
Plasma LH	foot shock	100 μg	B		195
	ether	100 μg		0	177
Physiological					
Plasma NE	ether	100 μg	0		39
Plasma EPI	ether	100 μg	B		39
Gastrointestinal function:					
Gastric acid secretion	surgery	10, 50 μg	B		227
	partial restraint	5–50 μg	B		144
Gastric emptying	surgery	?	B		239
	partial restraint	60 μg	B		144
	noise	200 ng	B	B (IP)	98
Bowel transit	partial restraint	50 μg	B		258
	partial restraint	50 μg	B		144
Behavioral					
Ingestion	restraint	50 μg	Att		134
Shock-induced freezing	foot shock	25 μg	B		124
Shock-induced fighting	shock	5, 25 μg	B		242
Exploratory behavior in the MCC	restraint	10–50 μg	B		17
Defensive withdrawal	novelty	1 μg			44, 240, 266
Conditioned emotional response		1, 5, 25 μg	Att		52
Acoustic startle	conditioned fear	5, 25 μg	B		234

expression of the IL-2 receptor[216]. In contrast to the lack of effect of CRF in vitro on NK activity mentioned above[117], Pawlikowski et al.[181] found that CRF (10^{-10}–10^{-6} M) inhibited NK activity. CRF also suppressed human peripheral leukocyte chemotaxis[228].

Kavelaars et al.[128] showed that CRF ($7 \cdot 10^{-8}$ M) in vitro stimulated β-endorphin secretion by human monocytes. Interestingly, this effect appears to be mediated by interleukin-1, suggesting that this cytokine may mediate some of the other in vitro effects of CRF[128]. In rats, CRF (0.1–10 μg SC) stimulated β-endorphin production by a small proportion of splenic and mesenteric lymph node lymphocytes[129].

7. INVOLVEMENT OF THE HPA AXIS IN THE EFFECTS OF CRF

Very few of the above-described effects can be attributed to activation of the HPA axis, i.e. secondary release of ACTH, endorphins or glucocorticoids. However, this possibility has been rigorously excluded in relatively a few cases. The relevant studies are listed in Table II.

Removal of the adrenal glands did not alter the effects of i.c.v. CRF on plasma glucose[38], mean arterial blood pressure and heart rate[85], nor on gastric emptying[143,238]. However, the effects of gastric acid secretion were blocked by adrenalectomy[66,236]. This result implicates plasma catecholamines or glucocorticoids in this particular response. In terms of the behavioral responses, the effects on active avoidance behavior were not affected by adrenalectomy[254].

In no case has hypophysectomy been shown to alter the effects of intracerebrally administered CRF. This was true for all of the physiological measures mentioned above and a wide variety of behavioral responses, including those on locomotor activity[75], feeding and grooming[163], active avoidance behavior[254] and exploration[20]. A functional hypophysectomy produced by dexamethasone administration to suppress pituitary secretion of ACTH did not alter the effects of i.c.v. CRF on mean arterial blood pressure and heart rate[85], locomotor activity[27,32], feeding[27], grooming[74,163] or in the Geller–Seifter conflict test[31].

Nevertheless, some of the effects of CRF may be due to secondary release of pituitary-adrenal hormones. Intracerebral administration of ACTH and β-endorphin induce grooming, so a secondary release of these peptide hormones within the brain cannot be excluded as a cause of this response to CRF. Indeed, the profile of the grooming response elicited by CRF closely resembles that for ACTH, suggesting that CRF-induced ACTH may be responsible for this effect[74]. Also, ACTH, β-endorphin and glucocorticoids are known to affect passive and active avoidance behavior[22,67], although in some cases opposite effects of the various hormones have been observed (see above).

It should also be noted that in a variety of instances, peripheral administration of CRF lacked the effects of central administration, while generally the former route of administration is more potent in activating the pituitary-adrenal system. Such instances include: LH secretion[188], GH secretion[127,189], locomotor activity[27], grooming behavior[27,211], several effects on passive and active avoidance behavior[254] and defensive withdrawal[240]. However, many of the gastrointestinal effects of CRF can be elicited by peripheral administration: gastric acid secretion[131,144,237]; gastric emptying[143,144,238,260]; increased large bowel transit[144,258]; migrating motor complexes[99] and antral activity[88].

8. INVOLVEMENT OF THE AUTONOMIC NERVOUS SYSTEM IN THE EFFECTS OF CRF

As reviewed above, i.c.v. CRF administration can activate the sympathetic nervous system. In general this occurs only at relatively high doses of CRF (≥ 1 μg). Thus there is a real possibility that responses observed at doses of CRF in this range may be due to sympathetic activation. Brown and Fisher showed that the ganglionic blocker, chlorisondamine, reversed or markedly attenuated the effects of i.c.v. CRF on the increases in plasma concentrations of NE, EPI and glucose[38] and those on heart rate and blood pressure[84,85]. Autonomic activation seems very likely to account for many of the gastrointestinal responses to i.c.v. CRF (gastric acid secretion, gastric emptying and gastrointestinal motility, see above) and may also account for some behavioral responses. The apparent mediation of the pyrogenic effects of IL-1β by CRF activation of the sympathetic nervous system[43,199] was also discussed above.

Britton and Indyk[28] investigated the possibility that the activation of the autonomic nervous system contributes to the locomotor-activating effects of CRF, using the ganglionic blockers chlorisondamine and hexamethonium. In the home cage, chlorisondamine but not hexamethonium, attenuated the CRF-induced (0.4 and 0.8 μg) increase in locomotion. In an open field, the CRF-induced decrease in locomotion and the increase in grooming were not affected by chlorisondamine but were attenuated by hexamethonium. Thus, these results suggest that an activation of the ANS could contribute to the actions of CRF on locomotor activity. By contrast, the CRF-induced decrease in food consumption was not significantly antagonized by either drug in either testing environment (except by chlorisondamine tested in the

home cage with 0.4 μg CRF). Thus, autonomic effects of CRF are not likely to contribute significantly to the ability of CRF to inhibit ingestive behavior. Neither hexamethonium nor chlorisondamine blocked the effect of CRF on the acoustic startle response[148]. However, both drugs tended to inhibit the CRF-induced increase in startle. The ability of i.c.v. CRF (1.0 μg) to diminish NK cell activity in the rat was abolished by chlorisondamine in parallel with its ability to prevent the increase in plasma NE[118].

The parasympathetic nervous system may also be involved in the effects of i.c.v. CRF. Several findings suggest that the vagus nerve plays a role in the effects of i.c.v. CRF on gastrointestinal functions (see above). Also, Fisher[83] reported that the i.c.v. CRF-induced change in the baroreflex control of heart rate was attenuated by atropine methyl nitrate, implicating a role for the parasympathetic nervous system (presumably the vagus) in this response. Thus the involvement of the autonomic system in the behavioral responses to i.c.v. CRF needs to be investigated further.

9. SITES OF ACTION OF CRF WITHIN THE BRAIN

Relatively little is known concerning where CRF acts within the brain to affect the variety of responses discussed above. Brown[34] studied the site(s) of action of the effects of CRF on plasma NE by injecting CRF (1 μg) into 50 different brain tissue sites. Some sites were responsive to CRF and some were not. When responses occurred at particular sites, they were not substantially greater than those obtained after intraventricular injections. A possible explanation is that the site of action of CRF is periventricular and that the tissue injections leak into the cerebral ventricular system.

The locomotor-activating effect of CRF (0.5 μg) injected into the substantia innominata/lateral preoptic region was significantly greater than that observed following injections of CRF into the pedunculopontine nucleus or frontal cortex[243]. An intermediate effect of CRF was observed when injected into the nucleus accumbens or the central nucleus of the amygdala[243]. However, in another study, injection of CRF (0.1 μg) into the amygdala of rats decreased locomotor activity in an open field[147]. Injection of CRF (0.06 μg) into the VTA increased locomotor activity in a photocell cage[125]. A similar effect was observed following higher doses i.c.v. (2–20 μg). In an open field, i.c.v. CRF (2 μg) increased the latency to move, but had no effect on locomotor activity, whereas 2 μg injected intra-VTA had no effect on latency to move but increased locomotor activity. It was concluded that the action of CRF on locomotor activity was not exerted in the VTA.

Injection of CRF (0.1 μg) into the amygdala of rats decreased locomotor activity in an open field and enhanced performance in the passive avoidance test[138,147]. However, because the effect of CRF injected into other sites within the brain was not examined, we cannot be certain that the amygdala was the active site or whether the CRF diffused from the amygdala to other brain sites. In a subsequent study in mice, Lee and Tsai[139] compared the locomotor-activating effects of CRF injected into the amygdala (0.02 μg each side), the dentate gyrus of the hippocampus (0.01 μg) and the caudate nucleus (0.05 μg). Injections into the caudate nucleus were ineffective, while the hippocampal injections were more potent than those in the amygdala. CRF (0.1 μg) injected into the amygdala immediately following passive avoidance training in rats increased retention 24 h and 1 week later[138]. A lower dose (0.01 μg) also facilitated retention in a sexually motivated appetitive test 24 h and 1 week post-training, but 0.1 μg impaired performance. Thus, although CRF injected into the amygdala affects performance in memory tests, there appears to be a difference in the sensitivity between appetitively and aversively motivated behaviors. Amygdaloid lesions impaired the CRF-induced increment in the startle response[148], but CRF injected into the amygdala did not alter the response[148], although injections of CRF into the parabrachial nucleus did (M. Davis, personal communication).

In an attempt to localize the anorectic effects of CRF, 0.5 μg of the peptide was injected into the PVN, lateral hypothalamus, ventromedial hypothalamus, globus pallidum or striatum of rats[136]. The only effective location was the PVN, in which CRF also enhanced grooming and movement. Injections of CRF into the mesencephalic gray, arcuate-ventromedial hypothalamus or the medial preoptic area inhibited lordosis behavior[218,219,223].

Two groups of investigators have used the cold cream blocking technique to determine where within the ventricular system CRF acts. Tazi et al.[243] found that 1 μg of CRF had a locomotor activating effect when injected either into the lateral ventricles or the cisterna magna. However, when the cerebral aqueduct was blocked by injection of cold cream, the latter site was no longer effective. These data in conjunction with their data on localized injections (see above) led the authors to conclude that the locomotor activating effect of CRF in a familiar environment involves an action of CRF in the ventral forebrain. We have investigated the effect of a similar block of the cerebral aqueduct on the ability of CRF (0.02 μg) to decrease exploratory behavior in the MCC. Blockade of the cerebral aqueduct prevented the decrease in exploration when CRF was injected into the 4th ventricle, but not the lateral ventricles[226]. Further,

blocking access of the peptide to the anteroventral quadrant of the third ventricle (AV3V) blocked the effect of lateral ventricle injection of CRF[226]. When other areas on the third ventricular surface (but not the AV3V) were blocked, the effect of CRF was not significantly attenuated. It is of interest that this region of the third ventricle was also determined to be a critical site in the ability of angiotensin to increase drinking[110], bradykinin to increase blood pressure[146] and ACTH to increase grooming[71].

10. INTERACTION OF CRF WITH OTHER NEUROTRANSMITTER SYSTEMS

Relatively little is known of the neurotransmitter systems with which CRF interacts to effect the responses described above.

10.1. Catecholamines

Van Loon et al. first investigated the effect of CRF on cerebral catecholamine metabolism in rats[252]. Using *m*-hydroxybenzylhydrazine to inhibit L-aromatic amino acid decarboxylase (a synthetic enzyme for catecholamines and serotonin (5-HT)), they failed to find any effects of IC CRF (5 μg) on the synthesis of 3, 4-dihydroxyphenylalanine (a precursor of dopamine, DA and NE) or 5-hydroxytryptophan (a precursor of 5-HT). Likewise, using the monoamine oxidase inhibitor, pargyline, to prevent degradation of catecholamines, CRF (20 μg IC) failed to alter the increased accumulation of DA, NE, EPI or 5-HT. They concluded that CRF did not alter the 'turnover' of cerebral catecholamines. However, Andersson et al.[7] studying the effects of i.v. CRF (100 μg/kg) in hypophysectomized rats found that the disappearance of NE (determined by histofluorescence) from the median eminence region was accelerated following inhibition of synthesis with α-methyl-*p*-tyrosine. Moreover, the disappearance of NE from the PVN was decreased. They concluded that the changes reflected regulatory feedback mechanisms and that NE most likely had a stimulatory effect on CRF-containing neurons in the PVN.

We have studied the effects of i.c.v.-administered CRF on the cerebral concentrations of catecholamines, indoleamines and their catabolites. CRF was administered into the lateral ventricles of mice and various brain regions sampled 30 min later. One μg of CRF significantly increased the concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC, a catabolite of DA) in the prefrontal cortex, nucleus accumbens, septum, striatum, hypothalamus and brain stem and of 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG, a catabolite of NE) in the prefrontal cortex, hypothalamus and brainstem of mice, without any significant changes in the parent catecholamines[69]. A lower dose of CRF (0.2 μg) had some of the same effects. Rather similar effects were observed using SC CRF administration at higher doses (1 or 10 μg); DOPAC was increased in prefrontal cortex and MHPG in prefrontal cortex, hypothalamus and brainstem. These effects of i.c.v. and SC administration of CRF resemble those observed with behavioral stressors such as footshock or restraint[68], except that no increases in brain tryptophan or in 5-HT metabolism were observed. The effects of i.c.v. CRF on DOPAC and MHPG were not altered by naloxone pretreatment (0.8 mg/kg)[69]. A recent report found very similar results in rats[156]. One μg CRF increased DOPAC:DA ratios in frontal cortex, nucleus accumbens, striatum and amygdala and 10 μg had similar effects in all these regions plus the hippocampus. MHPG:NE ratios were increased by both doses of CRF in frontal cortex and hippocampus.

Other authors have essentially confirmed CRF-induced increases in DA and NE metabolism in rats. Kalivas et al.[125] observed increases in DOPAC and homovanillic acid (HVA, another DA catabolite) in the prefrontal cortex, nucleus accumbens and striatum following i.c.v. administration of CRF (2 or 20 μg), although these effects were only statistically significant at the higher dose. By contrast, decreases in DOPAC and HVA were observed in the prefrontal cortex of rats following CRF administration (0.2 or 2.0 μg) into the VTA. Butler et al. found that 1 μg CRF injected into the locus coeruleus increased cerebral concentrations of 3,4-dihydroxyphenylethyleneglycol (DHPG, another catabolite of NE) in the amygdala and posterior hypothalamus 45 min later[44]. The minimum effective dose of CRF i.c.v. was 0.5 μg. However, similar changes were observed with lower doses of CRF (minimum effective dose 0.01 μg) when the peptide was applied locally in the region of the LC. The effects of i.c.v. CRF on MHPG and DHPG are consistent with the increased firing rate of LC neurons observed following i.c.v. administration of 1 μg CRF[250] (see above). The activation of cerebral biogenic amines has been confirmed in pigeons in which i.c.v. CRF (5.6–30 μg/kg) increased CSF concentrations of DOPAC and HVA, with lesser effects on MHPG[14].

The association of CRF administration with an activation of catecholamine metabolism is also consistent with reports that striatal tyrosine hydroxylase from rats or mice is activated by CRF (10^{-8}–10^{-6} M) in vitro[172,173].

We know that the release of CRF occurs during stress[187] and that catecholaminergic systems are activated under similar conditions[72]. It has long been known that NE plays a role in the regulation of CRF release[256], but the nature of this role has been confused. Anatomical studies have established that there is a direct noradren-

ergic input to the hypothalamic PVN[57]. A recent study provides good evidence for a dopaminergic input also[151]. Other studies have suggested catecholaminergic input to CRF-containing cells in other regions: the central nucleus of the amygdala, the bed nucleus of stria terminalis, but not the suprachiasmatic nucleus[113]. Recent evidence from several laboratories suggest that NE is largely stimulatory on CRF release, and that during stress noradrenergic terminals in the PVN stimulate CRF release through an α_1-receptor[3,184,235]. Al-Damluji[3] and Plotsky et al.[185] have reviewed the available evidence and provided plausible explanations of the previous interpretations.

This leaves us with the interesting situation that CRF can both activate and be activated by noradrenergic systems. Such a reciprocal relationship should perhaps not be surprising, considering that adapting to stress (i.e. coping) is a most important function for any living organism. It should perhaps be noted, however, that the activation of cerebral catecholaminergic systems occurs only at relatively high doses of CRF, higher than the minimal doses required to elicit several behavioral effects.

Although CRF may alter DA release, dopamine systems do not appear to be involved in the locomotor-activating effect of CRF observed in a familiar environment because α-flupenthixol (a dopamine receptor antagonist) did not antagonize the effect of 1 μg i.c.v. CRF except at cataleptic doses[132]. Likewise, haloperidol failed to reverse the effects of intra-VTA injections of CRF on locomotor activity[125]. Further, 6-hydroxydopamine lesions of the nucleus accumbens, which prevented the locomotor-activating effects of amphetamine, had no effect on the CRF-induced increase in locomotor activity[232]. Nevertheless, CRF (0.02 or 0.1 μg) potentiated amphetamine-induced stereotyped behavior in rats[51].

Noradrenergic systems might have a role in mediating the locomotor activating effect of CRF observed in a familiar environment. Chronic treatment with desmethylimipramine, a norepinephrine re-uptake inhibitor, enhanced the locomotor-activating effect of CRF[79]. Yohimbine, an α_2-antagonist (10 nmol) administered i.c.v. and phentolamine, an α-antagonist, (10 nmol) inhibited the CRF-induced increase in locomotor activity without affecting caffeine-induced increases in activity[114].

The β-adrenergic antagonist, propranolol, enhanced the CRF-induced increase in locomotor activity in a familiar testing environment[50]. In a conditioned emotional response, *l*-propranolol (but not *d*-propranolol) blocked the CRF-induced increased suppression of responding in the conditioned-stimulus, but not the preconditioned stimulus components[50]. This could indicate that β-adrenoceptors regulate stress-related behavioral responding. *l*-Propranolol (but not the *d*-form) reversed the restraint- and CRF-induced increases in defensive withdrawal behavior in rats[266]. This effect appears to be mediated by a central β_1-adrenoceptor[265]. Propranolol also blocked the CRF-induced decrease in the pentobarbital-induced sleeping time[115].

The effect of restraint on exploratory behavior of mice in the MCC appears to involve a NE-stimulated release of CRF[19]. Treatments that increase NE release including restraint[16] and the α_2-antagonist, idazoxan (1 mg/kg), decreased exploratory behavior[18,19]. In contrast, the α_2-agonist (clonidine 25 μg/kg, IP) or the noradrenergic-selective neurotoxin, DSP-4, increased exploration in unrestrained mice and antagonized the restraint-induced decrease in this response[19]. When combined, DSP-4 and clonidine completely blocked the effect of restraint stress[19]. The α_1-receptor-antagonist, prazosin (200 μg/kg), also prevented the behavioral effect of restraint, whereas the α_1-agonist, phenylephrine (50 or 100 ng i.c.v.) decreased exploratory behavior[19]. Because phenylephrine does not cross the blood–brain barrier readily and the dose administered in these studies was significantly lower than that at which cardiovascular effects are observed when administered peripherally, it is probable that central noradrenergic receptors are involved in these effects of phenylephrine. None of the above treatments consistently affected locomotor activity. Neither DSP-4 nor prazosin altered the CRF-induced decrease in exploration. However, the CRF-antagonist, ahCRF (20 μg), reversed the phenylephrine-induced decrease in exploratory behavior[19]. Rather similar results were obtained studying defensive withdrawal in rats. Prazosin or clonidine decrease defensive withdrawal in naive rats[266]. Restraint increased defensive withdrawal in rats familiar with the apparatus and this effect was prevented by prazosin or clonidine[266]. I.c.v. CRF also increased defensive withdrawal[44,240,266]. I.c.v. phenylephrine mimicked the effect of restraint or CRF[266]. Prazosin reversed the effect of phenylephrine but not CRF[266]. However, the effect of phenylephrine was reversed by i.c.v. ahCRF[266]. Thus in both these paradigms (exploratory behavior in mice and defensive withdrawal in rats) noradrenergic and CRF systems appear to interact such that noradrenergic systems regulate the release of endogenous CRF via an α_1-receptor. Such an arrangement parallels the organization of the noradrenergic and CRF systems instrumental in ACTH release[3,184,235].

10.2. Serotonin

In the studies with CRF discussed above, Van Loon did not find any changes of 5-HT metabolism related to i.c.v. injection of CRF[252] and we did not observe any changes in 5-HT or its catabolite, 5-hydroxyindoleacetic acid (5-HIAA) after i.c.v. infusion of 0.2 or 1.0 μg[69].

However, i.c.v. CRF (5.6–30 μg/kg) increased CSF concentrations of 5-HIAA in pigeons[14]. Moreover, a recent report indicated that 3 μg CRF administered i.c.v. to rats activated tryptophan hydroxylase assayed in vitro[214]. This effect of CRF was also obtained with infusions of CRF into the central amygdaloid nucleus[215]. Notably these effects were observed only at very high doses of CRF, so their physiological significance is unclear.

10.3. Acetylcholine

When injected together with carbachol (4 μg) into the medial frontal cortex in rats, CRF (0.001–0.01 μg) inhibited the carbachol-induced repetitive forepaw treading ('boxing')[55]. This result suggests that CRF may have anticholinergic properties. A higher dose of CRF (0.2 μg) injected alone into this region had no observable effects on behavior.

10.4. GABA

The effects of benzodiazepines on the responses to CRF (see above) provide indirect evidence for an interaction between CRF and GABAergic systems, but no direct evidence for an interaction between CRF and benzodiazepine receptors or benzodiazepines and CRF-receptors has been reported. Nevertheless, Sirinathsinghji and Heavens[221] found that CRF injected into the striatum and globus pallidum stimulated GABA release in vivo.

10.5. Endogenous opiates

A recent study found extensive colocalization of enkephalin- and irCRF in all regions of the PVN, the medial preoptic area, bed nucleus of the stria terminalis, periventricular hypothalamic nucleus, lateral and dorsal hypothalamic areas and subincertal nucleus[206]. Input of cells containing irACTH (which may thus also contain β-endorphin) to irCRF cells also occurs in the PVN and the bed nucleus of the stria terminalis[113]. CRF has been shown to possess a powerful ability to release endorphins both in vivo and in vitro. Sirinathsinghji et al.[222] showed that 10^{-12}–10^{-8} M CRF applied in 75 min pulses stimulated the release of both dynorphin and Met-enkephalin from striatal slices. Moreover, similar concentrations of CRF stimulated the release of both peptides from the caudate nucleus and globus pallidus in vivo. Both the in vivo and the in vitro effects were prevented by ahCRF (10^{-6} M). Thus some of the effects of CRF administration may be mediated by endogenous opiates.

The involvement of endogenous opiates in physiological and behavioral responses is normally determined by testing the sensitivity to the opiate antagonist, naloxone or its longer acting analog, naltrexone. However, more specificity can be obtained by the use of antisera to the various endogenous opioid peptides and this has been done in a few studies with CRF. The data on the ability of naloxone, naltrexone or β-endorphin antisera to antagonize the effects on CRF are summarized in Table III.

Conaglen et al.[53] reported that naloxone enhanced the ACTH, cortisol and aldosterone responses to i.v. CRF administration in man. Naloxone blocked the bradycardia, but not the hypotension caused by i.v. CRF in rats[130]. As discussed above, endogenous opiates are involved in other endocrine responses to CRF. Naloxone has been variously reported to reverse or attenuate the inhibitory effect of CRF on LH secretion[6,13,90,171], and on FSH secretion in primates[13,90]. It also prevented the elevation of prolactin secretion[253]. All of these effects involve peripheral effects of CRF. According to one report, naltrexone did not alter the decrease in LH secretion caused by i.c.v. CRF[188]. However, an attenuation of LH secretion in rats was also observed using i.c.v. antiserum to β-endorphin or dynorphin-A, but not to enkephalin[182].

As mentioned above, injections of CRF into the mesencephalic gray, arcuate-ventromedial area of the hypothalamus or the medial preoptic area inhibited lordosis behavior[218,219,223]. In the mesencephalic gray and arcuate-ventromedial area of the hypothalamus this effect of CRF was significantly inhibited or abolished by pretreatment of the tissue site with naloxone or antisera to β-endorphin, but not by antisera to dynorphin or Met-enkephalin[218,223]. In the medial preoptic area, CRF appears to act synergistically with β-endorphin to inhibit lordosis[219]. In both the mesencephalic gray and medial preoptic area the effect of CRF could be abolished with infusions of LHRH into these regions, whereas the facilitation of lordosis observed with anti-β-endorphin and CRF antisera were blocked by an LHRH antagonist. Thus, in these two regions CRF appears to inhibit lordosis by inhibiting the release of LHRH[218,219,223]. In male rats, naloxone infused into the third ventricle (10 μg) blocked the disruptive effect of CRF on sexual behavior[220].

In addition to that observed with sexual behavior, opiates might also be involved in some of the other behavioral effects of CRF. Naloxone reversed the CRF-induced decrease in exploratory behavior in mice at a dose that had no significant effect on this response in the absence of CRF[16]. Naloxone also blocked the i.c.v. CRF-induced increase in grooming behavior in rats[74]. However, naloxone (0.02–5.0 mg/kg) has been reported not to block the locomotor-activating effect of CRF[132,153], although, in a third study naloxone did block this effect of CRF[207]. The dose of naloxone used in the latter

study (presumably 3 mg/kg), could be considered high and the authors failed to exclude possible sedative effects of naloxone.

11. THE ROLE OF CRF IN STRESS-RELATED EFFECTS

The above review has detailed a large number of endocrine, neurochemical, electrophysiological and behavioral changes elicited by CRF. As pointed out earlier most of these mimic or are compatible with responses observed in stress[93,133]. Of critical significance are the activation of the pituitary-adrenal system, the sympathetic nervous system and adrenal medulla and cerebral catecholamines, all of which are regarded as primary components in the stress response[12,72]. Nevertheless, these results themselves could be interpreted to indicate that i.c.v. administration of CRF is stressful and thus elicits a range of responses commonly observed in stress. A crucial test is the ability of a CRF antagonist to prevent the changes observed in stressful situations. Fortunately, both CRF antisera and at least one peptide antagonist of CRF exists. α-Helical CRF_{9-41} appears to be relatively specific, although disappointingly high doses are needed to effectively block CRF-receptors[194] and because it is a peptide it does not readily cross the blood–brain barrier.

Thus far most reports have found CRF antagonists to attenuate or prevent stress-related changes (Table IV). I.c.v. ahCRF prevented the electric footshock-induced decreases in GH[191] and LH[195] secretion. Antibody to CRF blocked the ether exposure-induced decreases in GH but not LH secretion[177]. AhCRF also prevented the increase in plasma EPI, but not NE[39]. It also prevented the gastrointestinal effects of i.c.v. CRF (gastric acid secretion, gastric emptying and gastric motility) and antibody to CRF blocked the noise-induced increase in gastric emptying[98] (see above). As far as the behavioral responses are concerned, i.c.v. ahCRF attenuated the restraint-induced decrease in feeding[134], blocked the restraint-induced decrease in exploratory behavior in the MCC[17], the electric shock-induced increases in fighting[242] and freezing[122], attenuated the fear-induced exacerbation of acoustic startle[234] and decreased the acquisition of a CER[52]. In the defensive withdrawal task, it decreased withdrawal in a novel open field[240] and reversed the restraint-induced increases in withdrawal in rats familiar with the apparatus[266]. These results give extraordinary cogency to the argument that CRF is an endogenous mediator of these responses.

A priori, it seems unlikely that i.c.v.-injected CRF is able to reach all cerebral CRF-receptors in sufficient concentration to activate them, but this may be the reason for the high doses of CRF necessary to elicit some of the stress-like responses. However, if, as Ono et al.[177] have suggested, i.c.v. CRF can activate endogenous CRF systems, there is a mechanism for a global activation of CRF receptors. An alternative possibility is that high doses of CRF specifically activate catecholaminergic systems that in turn elicit release of endogenous CRF. The uniformity of the results with the CRF antagonists is truly remarkable given the probable difficulty in obtaining adequate concentrations at the appropriate brain sites. Indeed it would not have been surprising if a variety of stress-related responses observed following CRF administration, were not affected by the antagonist. On the other hand, brain sites accessible to CRF are probably accessible to ahCRF, because the latter peptide is less hydrophilic.

A role for CRF in anxiety or stress is supported by the effects of benzodiazepines. In a number of cases, benzodiazepines elicited behavioral effects opposite to those following administration of CRF: feeding in an open field[24–26]; punished responding in the Geller–Seifter conflict test[30,33]; social interaction[70]; and defensive withdrawal[266]. In several cases, benzodiazepines antagonized or reversed the effects of CRF: locomotor activation by low doses of CRF in an open field[140]; suppression of punished responding in the Geller–Seifter test[30,33]; decreases in social interaction[70]; enhancement of acoustic startle[233]; and increased defensive withdrawal[266]. Moreover, the anxiogenic benzodiazepine inverse agonist, FG 7142, like CRF, decreased punished responding in the Geller–Seifter test[33]. These results suggest that CRF can be anxiogenic, so that endogenous CRF may be a mediator of anxiety. Experimentally, we are poorly equipped to distinguish anxiety from stress in animal studies.

12. CONCLUSIONS

The foregoing indicates that CRF can elicit a number of responses normally regarded as characteristic of anxiety or stress. The list includes many if not most of the responses symptomatic of stress. Because CRF antagonists are able to attenuate or reverse the effects of various stressors, CRF appears to be a mediator of these responses. Because the responses to intracerebrally administered CRF cover the entire spectrum of responses observed in stress, it is possible to postulate that the secretion of brain CRF may be both necessary and sufficient to define stress.

Postulating a role for cerebral CRF in stress suggests that the functions of CRF in the brain are akin to those involved in the activation of the HPA axis. This hypothesis is not unreasonable, but we know very little of the mechanisms regulating the release of CRF from cells outside the PVN. There is good evidence that activation

of noradrenergic systems in stress is the primary mechanism responsible for the release of CRF from neurons in the PVN, possibly through an α_1-receptor, although undoubtedly other neurotransmitter systems (e.g. acetylcholine, 5-HT and GABA) are also involved. We do not know whether similar mechanisms operate for other CRF-containing cells in the brain. It is possible that CRF-containing neurons in the PVN have collaterals reaching to widespread areas of the brain. However, the existing anatomical evidence for CRF-containing cell bodies in extrahypothalamic regions suggests that this is not the case. An interesting alternative would be the innervation of CRF-containing cells by the network of catecholaminergic terminals in widespread areas of the brain, including the cortex. Only further detailed research on the inputs to CRF-containing neurons can resolve this issue.

Interestingly, a CRF hypothesis of stress provides support for Selye's ideas of non-specificity in stress. Selye conceived this hypothesis because he considered the sympathoadrenal and HPA responses to be common to all stressors. Although Mason[155] and others have criticized the non-specificity concept, it is generally agreed that both catecholamine and HPA systems are activated in most, if not all, situations commonly regarded as stressful. We conceive the primary response in stress to be the activation of cerebral noradrenergic systems, which in turn activates CRF secretion and hence activates the HPA axis. This cerebral noradrenergic activation may also be responsible for the activation of the autonomic nervous system, perhaps via descending tracts originating in the PVN. Alternatively, at high rates of CRF secretion (corresponding to high doses of CRF), CRF may directly activate the autonomic nervous system and the adrenal medulla via descending tracts. Such a hypothesis would explain the coactivation of catecholamine and CRF systems in stress and many of the data reviewed above. It may well be that combined activity of catecholamine and CRF systems is necessary to manifest all aspects of stress. For example, the direct effects of NE on cerebral neurons to increase signal-to-noise ratios may provide a mechanism for selective attention. This effect of NE would then complement independent actions of CRF, which may induce arousal or even fear.

12.1. The effect of CRF dose

The question of peptide dose is always delicate, because peptides are generally considered to be metabolically labile and because their access to specific brain sites following intracerebroventricular administration may be limited by their size[149]. Nevertheless, because so many of the studies of intracerebrally administered CRF have been performed with lateral ventricle injections it is permissible to make comparisons. Several effects of i.c.v. CRF exhibit biphasic responses. Thus in a novel environment, low doses of CRF increased locomotor activity (0.01 μg[230,254] or 0.2 μg[140]), whereas high doses ($\geq$ 1 μg) decreased it[25,26,114,140,230,254]. Likewise, lower doses of CRF (0.1 μg) increased feeding in food-deprived rats, whereas higher doses (5 μg) decreased it[94]. Also, lower doses of CRF (0.01 and 0.1 μg) increased shock-induced boxing and fighting, but 1 μg disrupted the behavioral responding[242]. In the multicompartment chamber, we observed that mice that had received doses of CRF greater than about 0.15 μg into the lateral ventricles displayed abnormal behavior, characterized by prolonged periods of inactivity. Similar effects were observed in some rats with 0.05 μg and most at 0.1 μg[226]. It is possible in some circumstances that this response may reflect seizure activity which has been observed at doses in this range[77].

We suggest that the behavioral and physiological responses to i.c.v. CRF can be divided into high-dose and low-dose effects. The high-dose effects include the activation of the sympathetic nervous system and the adrenal medulla and of cerebral catecholamines. Such effects likely explain the gastrointestinal effects of i.c.v. CRF and may be associated with behavioral responses to higher doses of CRF, such as the decreased feeding and sexual behavior, the decreased locomotor activity in a novel environment, the increased locomotor activity in a familiar environment, the anxiogenic effects in the Geller–Seifter conflict test and the exacerbated acoustic startle response. On the other hand, the enhancement of locomotor behavior in a novel environment, decreased social interaction, exploratory behavior in the MCC, defensive withdrawal and increased feeding and shock-induced fighting are observed at considerably lower doses (0.005–0.1 μg).

Curiously, both the high-dose and the low-dose effects of CRF may be specific in the sense that ahCRF has the ability to reverse the CRF-induced changes. This is true for the endocrine[191,195] and gastrointestinal changes[98,144,227,239,260] as well as the activation of the adrenal medulla[39], the increase of locomotor activity in a familiar environment and the decreased responding in the Geller–Seifter test[32].

Distinctions between the low- and high-dose effects of CRF are not readily made on the basis of their pharmacological characteristics. For example, the opioid antagonist, naloxone blocked the effects of CRF on exploratory behavior[16] and grooming[74]. However, whereas naloxone can prevent the effects of CRF on sexual behavior[220], it failed to alter the locomotor-activating effect of CRF[132,153] and had rather complex effects on the CRF-induced changes in endocrine and gastrointestinal

functions (see Table III), all high-dose effects. The same is true for β-adrenergic antagonists. Propranolol prevented the effects of CRF on conditioned emotional responses, but enhanced the CRF-induced increase in locomotor activity in a familiar environment[50]. However, propranolol reversed the effects of CRF on defensive withdrawal in rats[265,266].

What is the significance of the two sets of dose effects of CRF? It is possible that they represent two distinct degrees of stress. The low-dose effects may correspond to mild or moderate activations of noradrenergic systems such as may be associated with arousal and induce a state of mild anxiety. The higher doses of CRF may directly activate LC noradrenergic neurons and perhaps also dopaminergic systems. Thus the responses to high doses of CRF may reflect the combined effects of both CRF and catecholamines. The noradrenergic activation caused by high doses of CRF may provoke release of endogenous CRF, forming a positive feedback loop, escalating the release of both catecholamines and CRF. In the absence of exogenous CRF, a similar state may perhaps be achieved by prolonged or intense activation of the noradrenergic systems, such that the amounts of CRF released could be sufficient to activate catecholaminergic systems, and thus provide a positive feedback, stimulating the release of still more CRF. Such a situation may be akin to panic. It would be associated with powerful activation of both cerebral catecholaminergic systems and the autonomic nervous system, as well as cerebral CRF systems. Do two different kinds of CRF-receptor mediate the effects of low and high doses or is the difference one of access to appropriate sites? The data obtained with presently available antagonists, which can block both high- and low-dose effects, suggest the latter. However, more careful studies using a variety of CRF-antagonists may suggest the involvement of different types of receptor.

12.2. *Clinical relevance*

A number of observations have suggested that CRF functions abnormally in depressed patients. The response of plasma ACTH to CRF administration has been shown to be blunted[93,111]. Subsequently, Nemeroff et al.[167] reported that the CSF concentrations of CRF were significantly elevated over normals in depressed patients. This result has subsequently been confirmed by the same group[168]. Such a result is consistent with the long standing observation of elevated plasma cortisol and an insensitivity to dexamethasone[168,225]. More recently, depressed suicide victims have been found to exhibit a decreased number of CRF-binding sites in the prefrontal cortex[169]. These relationships all suggest that the secretion of brain CRF is elevated in depression, and that this elevation leads to a desensitization of CRF receptors. This is not to say that depression is *caused* by an abnormality in CRF secretion. However, given the animal data reviewed above, it is not unlikely that some of the symptoms of depression may be related to the hypersecretion of CRF.

The link between noradrenergic systems and CRF discerned for exploratory behavior in the MCC and possibly in defensive withdrawal, is remarkable in the context of depression. For years the catecholamine hypothesis of depression has dominated experimentation in biological psychiatry. Recently, the hypothesis has been inverted, and it is now believed that depressed patients may exhibit hypersecretion of NE rather than the hyposecretion originally postulated. This position is reinforced by data from animal models of depression[257]. Again, it is not necessarily true that a hyperactivity of noradrenergic systems *causes* depression, but a CRF-noradrenergic interaction might be involved. The concept that hyperactivity in cerebral noradrenergic systems may stimulate hypersecretion of CRF reconciles the two hypotheses. In other words, the new catecholamine and the CRF hypotheses are not distinct, but merely reflect sequential steps in the same chain. If this is true, therapies for depression may be based not only on forcing down-regulation of noradrenergic systems, but also on antagonism of CRF.

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